cence or Jerusalem artichoke according to the technique described in the refs [12, 14, 15]. They were then chromatographed on a Sephadex-G 75 column. The active fractions were pooled and chromatographed on DEAE-sepharose equilibrated with NaPi (50 mM, pH 7.2) containing mercaptoethanol (10 mM); the proteins were eluted from the column by increasing concentrations of NaCl. The protein content of eluted fractions was determined by the method of ref. [16].

Isolation of intracellular membranes. Mitochondria or microsomes containing ^{32}P-labelled phospholipids were isolated as previously described [12] and stored at −80°. The protein content was determined by the biuret method [17].

Preparation of radioactive liposomes. Thin slices of tissue were incubated in $Na^{32}Pi$ (10 μCi/ml) or in [1-^{14}C]-NaOAc (15 μCi/ml) or in choline-[^{3}H] (5 μCi/ml) for 16 hr at 25°. After extraction of total lipids by the method of ref. [18], the phospholipids were separated from the other components by TLC [19] and eluted from the plates by $CHCl_3$–MeOH–H_2O (16·7:1). The phospholipids were dissolved in C_6H_6–EtOH (4:1) when storage was necessary. To prepare liposomes, the solvent was evapd and small glass beads were introduced in the flask [20]; after addition of exchange medium: sucrose (0.25 M), NaPi (50 mM, pH 7.2) and mercaptoethanol (10 mM), the soln was shaken 3 min with a vortex-mixer, then sonicated 30 min at 0° under N_2. A centrifugation for 1 hr at 20000 *g* gave a clear soln of liposomes.

Exchange assays. All expts were performed at 30° for 15 or 30 min; the total vol. was 4 ml. Unlabelled mitochondria (3 mg protein) and radioactive microsomal fraction (1 mg protein) or labelled liposomes were incubated in the exchange medium described above, with or without phospholipid exchange proteins. After incubation, the mitochondria were pelleted by centrigugation at 15000 *g* for 10 min and washed twice. The lipids of the mitochondria and supernatant were then extracted [18]. Phospholipids phosphorus was determined by the method of ref. [21].

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ACTIVATION OF CHLOROPLAST ATPase BY REDUCED THIOREDOXIN

DAVID W. MCKINNEY, BOB B. BUCHANAN and RICARDO A. WOLOSIUK
Department of Cell Physiology, University of California, Berkeley, CA 94720, U.S.A.

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Abstract—Chloroplast thioredoxin that is reduced by dithiothreitol activates the ATPase that is associated with solubilized preparations of chloroplast coupling factor (CF_1).

We have recently described a mechanism of light-actuated enzyme regulation that depends on two newly identified soluble chloroplast proteins [1–4]. One of these new proteins, ferredoxin-thioredoxin reductase, catalyses the reduction of the second protein, thioredoxin, in a reaction that utilizes photoreduced ferredoxin (equations 1 and 2).

$$H_2O + 2\ \text{Ferredoxin}_{\text{oxidized}} \xrightarrow[\text{chloroplast membranes}]{\text{light}} 2\ \text{Ferredoxin}_{\text{reduced}} + \tfrac{1}{2}O_2 \quad (1)$$

$$2\ \text{Ferredoxin}_{\text{reduced}} + \text{Thioredoxin}_{\text{oxidized}} \xrightarrow[\text{reductase}]{\text{Ferredoxin-thioredoxin}} \text{Thioredoxin}_{\text{reduced}} + 2\ \text{Ferredoxin}_{\text{oxidized}} \quad (2)$$

The thioredoxin reduced in this manner is used for the activation of soluble regulatory enzymes of chloroplasts (equation 3).

$$\text{Enzyme}_{\text{inactive}} \xrightarrow[\text{thioredoxin}]{\text{reduced}} \text{Enzyme}_{\text{active}} \quad (3)$$

Enzymes activated by the ferredoxin–thioredoxin system can be deactivated in the dark by oxidants such as oxidized glutathione and dehydroascorbic acid [3] or, in the case of NADP-malate dehydrogenase, by an unidentified membrane-bound oxidant [4].

The thioredoxin needed for enzyme activation in equation 3 can be reduced experimentally in the dark with the nonphysiological sulfhydryl reagent dithiothreitol [1–4] (equation 4).

$$\text{Thioredoxin}_{\text{oxidized}} + \text{Dithiothreitol}_{\text{reduced}} \rightarrow \text{Thioredoxin}_{\text{reduced}} + \text{Dithiothreitol}_{\text{oxidized}} \quad (4)$$

With dithiothreitol, neither ferredoxin nor ferredoxin-thioredoxin reductase is needed for enzyme activation by thioredoxin [3–5]. In the present study, dithiothreitol-reduced thioredoxin was found to increase the reductant-dependent ATPase activity [6] that is associated with solubilized preparations of chloroplast coupling factor (CF_1) [7].

For most experiments, CF_1 was isolated from chloroplast membranes that were prepared and sucrose-extracted as described by Strotmann *et al.* [8]. This procedure gave reproducible preparations of the enzyme, as measured by the ability of a heated (4 min, 60°) preparation to catalyze the release of P_i from ATP in the presence of dithiothreitol and a divalent cation [9].

Table 1 shows that thioredoxin increased up to 2-fold the activity of ATPase with either Ca^{2+} or Mg^{2+} as divalent cation. Activation by reduced thioredoxin was observed at cation concentrations ranging from 0.5 to 10 mM, but the magnitude of activation was usually greater at a limiting cation concentration (Table 2). In this respect, the ATPase resembles chloroplast fructose 1,6-bisphosphatase—a regulatory enzyme of carbon dioxide assimilation—that also gives its most marked response to reduced thioredoxin at limiting cation (in this case, Mg^{2+}) concentrations [5, 10]. A further resemblance of CF_1 ATPase to the fructose 1,6-bisphosphatase was revealed by the current finding that dithiothreitol could not be replaced by sulfhydryl reagents such as reduced glutathione [5] in activation of the ATPase by thioredoxin.

Table 1. Activation of chloroplast ATPase by reduced thioredoxin

Treatment	P_i released, μmol	
	$MgCl_2$ as cation	$CaCl_2$ as cation
Complete	0.6	0.3
Minus thioredoxin	0.3	0.2
Minus dithiothreitol	0.3	0.1
Minus divalent cation	0.0	0.0

The complete system contained 25 μg of chloroplast thioredoxin [3], heat-activated CF_1 equivalent to 0.15 mg of chlorophyll [7], and the following (μmol): tricine buffer (pH 8), 50; ATP (pH 7.6), 5; dithiothreitol, 2.5; $MgCl_2$ or $CaCl_2$, 0.5. The final vol. was 0.5 ml. After adding all components except ATP, the reaction mixture was preincubated for 5 min; ATP was then added and the reaction was continued for another 20 min. Temp. 25°. The reaction was stopped by the addition of 2 ml of the mixture used for P_i analysis [10]. Prior to assay, CF_1 was activated by heating the enzyme in 13 mM ATP for 4 min at 60°.

Table 2. Effect of cation concentration on activation of chloroplast ATPase by reduced thioredoxin

Cation	Concentration (mM)	P_i released, μmol	
		Dithiothreitol	Dithiothreitol + Thioredoxin
$MgCl_2$	0.0	0.0	0.0
	0.5	0.05	0.1
	1.0	0.1	0.2
	5.0	0.3	0.4
	10.0	0.4	0.6
	20.0	0.4	0.8
$CaCl_2$	0.0	0.0	0.0
	0.5	0.04	0.1
	1.0	0.1	0.2
	5.0	0.3	0.5
	10.0	0.5	0.9
	20.0	0.5	0.9

Except for varying the concentration of divalent cation and adding dithiothreitol and thioredoxin as indicated, experimental conditions were as given in Table 1.

Apart from revealing certain common properties between the two phosphatases, the present study provides evidence that the capacity for activation by reduced thioredoxin is not peculiar to a particular preparation of the ATPase enzyme. ATPase that was solubilized by EDTA [9] showed a response to reduced thioredoxin similar to that of the sucrose-solubilized preparation in Table 1. A positive response to reduced thioredoxin was also observed with the sucrose-solubilized preparation that had been chromatographed (at 22°) on a BioGel A 1.5 m column developed in a solution of 50 mM Tris buffer (pH 7.9) and 50 mM NaCl.

The present results provide evidence that chloroplast thioredoxin can interact with a solubilized membrane-bound component of chloroplasts. The physiological significance of this interaction remains to be determined.

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